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Swiss-Prot: Q64280

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ID   TGF4_MOUSE     STANDARD;      PRT;   368 AA.
AC   Q64280;
DT   01-NOV-1997 (Rel. 35, Created)
DT   01-NOV-1997 (Rel. 35, Last sequence update)
DT   16-OCT-2001 (Rel. 40, Last annotation update)
DE   Transforming growth factor beta 4 precursor (TGF-beta 4) (Lefty
DE   protein) (Lefty-1 protein) (STRA3 protein).
GN   EBAF OR TGFB4 OR STRA3 OR LEFTY OR LEFTY1.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=96202359; PubMed=8610011; [NCBI, ExPASy, EBI, Israel, Japan]
RA   Meno C., Saijoh Y., Fujii H., Ikeda M., Yokoyama T., Yokoyama M.,
RA   Toyoda Y., Hamada H.;
RT   "Left-right asymmetric expression of the TGF beta-family member lefty
RT   in mouse embryos.";
RL   Nature 381:151-155(1996).
RN   [2]
RP   SEQUENCE FROM N.A.
RA   Bouillet P.;
RL   Submitted (JUN-1996) to the EMBL/GenBank/DDBJ databases.
RN   [3]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98156497; PubMed=9496783; [NCBI, ExPASy, EBI, Israel, Japan]
RA   Oulad-Abdelghani M., Chazaud C., Bouillet P., Mattei M.-G., Dolle P.,
RA   Chambon P.;
RT   "Stra3/lefty, a retinoic acid-inducible novel member of the
RT   transforming growth factor-beta superfamily.";
RL   Int. J. Dev. Biol. 42:23-32(1998).
RN   [4]
RP   FUNCTION.
RX   MEDLINE=98372436; PubMed=9708731; [NCBI, ExPASy, EBI, Israel, Japan]
RA   Meno C., Shimono A., Saijoh Y., Yashiro K., Mochida K., Ohishi S.,
RA   Noji S., Kondoh H., Hamada H.;
RT   "Lefty-1 is required for left-right determination as a regulator of
RT   lefty-2 and nodal.";
RL   Cell 94:287-297(1998).
CC   -!- FUNCTION: REQUIRED FOR LEFT-RIGHT AXIS DETERMINATION AS A
CC       REGULATOR OF LEFT2 AND NODAL.
CC   -!- SUBCELLULAR LOCATION: Secreted.
```

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CC   -!- DEVELOPMENTAL STAGE: BY E8.0, EXPRESSED EXCLUSIVELY ON THE LEFT
CC       SIDE OF DEVELOPING EMBRYOS WITH EXPRESSION PREDOMINANTLY IN THE
CC       PROSPECTIVE FLOOR PLATE (PFP). WEAK EXPRESSION IN THE LATERAL-
CC       PLATE MESODERM (LPM).
CC   -!- PTM: THE PROCESSING OF THE PROTEIN MAY ALSO OCCUR AT THE SECOND R-
CC       X-X-R SITE LOCATED AT AA 132-135. PROCESSING APPEARS TO BE
CC       REGULATED IN A CELL-TYPE SPECIFIC MANNER.
CC   -!- SIMILARITY: BELONGS TO THE TGF-BETA FAMILY.
CC   ---------------------------------------------------------------------
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CC   ---------------------------------------------------------------------
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DR   EMBL; Z73151; CAA97497.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
DR   EMBL; AJ000082; CAA03909.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
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DR   MGD; MGI:107405; Ebaf.
DR   GeneLynx; Ebaf.
DR   Ensembl; Q64280. [Entry / Contig view]
DR   SOURCE; Ebaf.
DR   InterPro; IPR001839; TGFb.
DR   InterPro; IPR001111; TGFb_N.
DR   InterPro; Graphical view of domain structure.
DR   Pfam; PF00019; TGF-beta; 1.
DR   Pfam; PF00688; TGFb_propeptide; 1.
DR   ProDom; PD000357; TGFb; 1.
DR   ProDom [Domain structure / List of seq. sharing at least 1 domain ]
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DR   PROSITE; PS00250; TGF_BETA_1; 1.
DR   BLOCKS; Q64280.
DR   ProtoNet; Q64280.
DR   ProtoMap; Q64280.
DR   PRESAGE; Q64280.
DR   DIP; Q64280.
DR   ModBase; Q64280.
DR   SWISS-2DPAGE; GET REGION ON 2D PAGE.
KW   Developmental protein; Growth factor; Cytokine; Glycoprotein; Signal.
FT   SIGNAL        1     21       POTENTIAL.
FT   PROPEP       22     76       OR 135 (POTENTIAL).
FT   CHAIN        77    368       TRANSFORMING GROWTH FACTOR BETA 4.
FT   DISULFID    253    266       BY SIMILARITY.
FT   DISULFID    265    318       BY SIMILARITY.
FT   DISULFID    295    353       BY SIMILARITY.
FT   DISULFID    299    355       BY SIMILARITY.
FT   CARBOHYD    158    158       N-LINKED (GLCNAC...) (POTENTIAL).
SQ   SEQUENCE   368 AA;  41497 MW;  821DAE663C546B5F CRC64;
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//
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Direct BLAST submission at NCBI (Bethesda, USA)

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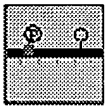
Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)



Feature table viewer (Java)

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[General] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

General information about the entry

Entry name	**TGF4_MOUSE**
Primary accession number	**Q64280**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 35, November 1997
Sequence was last modified in	Release 35, November 1997
Annotations were last modified in	Release 40, October 2001
Name and origin of the protein	
Protein name	**Transforming growth factor beta 4 [Precursor]**
Synonyms	**TGF-beta 4** **Lefty protein** **Lefty-1 protein** **STRA3 protein**
Gene name	**EBAF** or **TGFB4** or **STRA3** or **LEFTY** or **LEFTY1**
From	Mus musculus (Mouse) [TaxID: 10090]
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
References	

[1]	SEQUENCE FROM NUCLEIC ACID. MEDLINE=96202359; PubMed=8610011; [NCBI, ExPASy, EBI, Israel, Japan] Meno C., Saijoh Y., Fujii H., Ikeda M., Yokoyama T., Yokoyama M., Toyoda Y., Hamada H.; "Left-right asymmetric expression of the TGF beta-family member lefty in mouse embryos."; Nature 381:151-155(1996).
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[4]	FUNCTION. MEDLINE=98372436; PubMed=9708731; [NCBI, ExPASy, EBI, Israel, Japan] Meno C., Shimono A., Saijoh Y., Yashiro K., Mochida K., Ohishi S., Noji S., Kondoh H., Hamada H.; "Lefty-1 is required for left-right determination as a regulator of lefty-2 and nodal."; Cell 94:287-297(1998).

Comments

- ***FUNCTION***: REQUIRED FOR LEFT-RIGHT AXIS DETERMINATION AS A REGULATOR OF LEFT2 AND NODAL.
- ***SUBCELLULAR LOCATION***: Secreted.
- ***DEVELOPMENTAL STAGE***: BY E8.0, EXPRESSED EXCLUSIVELY ON THE LEFT SIDE OF DEVELOPING EMBRYOS WITH EXPRESSION PREDOMINANTLY IN THE PROSPECTIVE FLOOR PLATE (PFP). WEAK

EXPRESSION IN THE LATERAL-PLATE MESODERM (LPM).

- ***PTM***: THE PROCESSING OF THE PROTEIN MAY ALSO OCCUR AT THE SECOND R-X-X-R SITE LOCATED AT AA 132-135. PROCESSING APPEARS TO BE REGULATED IN A CELL-TYPE SPECIFIC MANNER.
- ***SIMILARITY***: BELONGS TO THE TGF-BETA FAMILY.

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Cross-references

EMBL	D83921; BAA12121.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] Z73151; CAA97497.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] AJ000082; CAA03909.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] AJ000083; CAA03910.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
HSSP	P10600; 1TGJ. [HSSP ENTRY / PDB]
MGD	MGI:107405; Ebaf.
GeneLynx	EBAF; Mus musculus.
SOURCE	EBAF; Mus musculus.
Ensembl	Q64280; Mus musculus. [Entry / Contig view]
InterPro	IPR001839; TGFb. IPR001111; TGFb_N. Graphical view of domain structure.
Pfam	PF00019; TGF-beta; 1. PF00688; TGFb_propeptide; 1.
ProDom	PD000357; TGFb; 1. [Domain structure / List of seq. sharing at least 1 domain].
SMART	SM00204; TGFB; 1.
PROSITE	PS00250; TGF_BETA_1; 1.

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ProtoNet	Q64280.
ProtoMap	Q64280.
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ModBase	Q64280.
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Keywords

Developmental protein; Growth factor; Cytokine; Glycoprotein; Signal.

Features

Key	From	To	Length	Description
SIGNAL	1	21	21	*POTENTIAL.*
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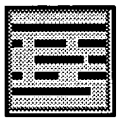
CHAIN	77	368	292	TRANSFORMING GROWTH FACTOR BETA 4.
DISULFID	253	266		*BY SIMILARITY.*
DISULFID	265	318		*BY SIMILARITY.*
DISULFID	295	353		*BY SIMILARITY.*
DISULFID	299	355		*BY SIMILARITY.*
CARBOHYD	158	158		N-LINKED (GLCNAC...) (*POTENTIAL*).



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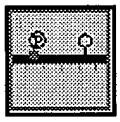

Feature table viewer

Sequence information

Length: **368 AA** [This is the length of the unprocessed precursor]	Molecular weight: **41497 Da** [This is the MW of the unprocessed precursor]	CRC64: **821DAE663C546B5F** [This is a checksum on the sequence]

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LIPRRLQP
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Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

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[General] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

General information about the entry

Patients with Turner's syndrome may have an inherent endometrial abnormality affecting receptivity in oocyte donation.
Yaron Y; Ochshorn Y; Amit A; Yovel I; Kogosowki A; Lessing JB
Serlin Maternity Hospital, Sourasky Medical Center, Tel Aviv, Israel.
Fertil Steril (UNITED STATES) Jun 1996 , 65 (6) p1249-52, ISSN 0015-0282 Journal Code: EVF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9609
Subfile: INDEX MEDICUS

253431

Implantation: from basics to the clinic.
Tabibzadeh S
Department of Pathology, University of South Florida, Tampa 33612, USA. tabibzadeh@moffitt.usf.edu
Ann N Y Acad Sci (UNITED STATES) Sep 26 1997, 828 p131-6, ISSN 0077-8923 Journal Code: 5NM
Contract/Grant No.: CA 56866, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
JOURNAL ANNOUNCEMENT: 9801
Subfile: INDEX MEDICUS
(43 Refs.)
Tags: Animal; Female; Human; Support, U.S. Gov't, P.H.S.

The progressive rise in the expression of alpha crystallin B chain in human endometrium is initiated during the implantation window: modulation of gene expression by steroid hormones.
Gruidl M; Buyuksal A; Babaknia A; Fazleabas AT; Sivarajah S; Satyaswaroop PG; Tabibzadeh S
Department of Pathology, Moffitt Cancer Center, Tampa, FL 33612, USA.
Mol Hum Reprod (ENGLAND) Apr 1997, 3 (4) p333-42, ISSN 1360-9947 Journal Code: CWO
Contract/Grant No.: CA46866, CA, NCI; CA62211, CA, NCI; HD29964, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE
JOURNAL ANNOUNCEMENT: 9711
Subfile: INDEX MEDICUS

Parker

The signals and molecular pathways involved in human menstruation, a unique process of tissue destruction and remodelling.
Tabibzadeh S
Dept of Pathology, University of South Florida, Tampa, USA.
Mol Hum Reprod (ENGLAND) Feb 1996, 2 (2) p77-92, ISSN 1360-9947 Journal Code: CWO
Contract/Grant No.: CA 56866, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC
JOURNAL ANNOUNCEMENT: 9711
Subfile: INDEX MEDICUS

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29/09906445544

Molecular Human Reproduction vol.2 no.2 pp. 77–92, 1996

REVIEW

Th signals and mol cular pathways involv d in human menstruation, a uniqu proc ss of tissu d struction and remod lling

S.Tabibzadeh

Dept of Pathology, University of South Florida, and Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612, USA

Human endometrium is a specialized tissue that undergoes sequential phases of proliferation and secr t ry changes in order to support the implantation and growth of an embryo. If implantation does not occur, this tissue rapidly undergoes dissolution during the menstrual period. Tissue shedding during menstruati n is associated with significant apoptosis, disordered expression of adhesion molecules, loss of filament us (F) actin from cell borders and fragmentation of endometrial glands. On the other hand, compromise of int grity of vessels and dissolution of the extracellular matrix leads to bleeding and tissue dissolution. The pr c ss s of bleeding and tissue shedding during menstruation are precisely controlled by a number of systemic and l cal factors. The systemic signal that leads to menstruation is the withdrawal of the steroid hormones. Th available evidence suggests but does not yet prove that tumour necrosis factor (TNF)-α may serve as th l cal signal contributing to the processes of menstrual shedding and bleeding. Secretion of metalloprot inases and their subsequent activation induced by plasmin facilitates degradation of extracellular matrices and bleeding. The menstrual process ceases by secretion of steroid hormones directly or through regulati n of pr duction or activation of signals that lead to tissue shedding and bleeding.

Key words: apoptosis/ epithelial cells/proliferation/steroid hormones/TNF-α

Distinct features of endometrium during the menstrual phase

In order to be prepared for implantation, the endometrium undergoes predictable, sequential phases of proliferation and secretory changes. However, if implantation does not occur, endometrial tissue is shed. After cessation of bleeding and tissue shedding, the integrity of the denuded endometrium is restored by a wave of repair activity. A number of features characterize human endometrium that has failed to bear an embryo. Impending menstruation is characterized by infiltration of endometrial tissue by the so-called stromal granulocytes thought to be equivalent to large granular lymphocytes (Bulmer, 1994). During menstruation, epithelial damage that consists of apoptosis, disordered localization of adhesion molecules, and loss of filamentous (F) actin from cell borders are likely to contribute to the glandular fragmentation. During this period, endothelial damage due to compromise in vessel integrity manifests as bleeding. These features of menstrual endometrium are discussed below.

Menstruation is associated with significant apoptosis in endometrial epithelium

A number of changes occur during the secretory phase that forecast an impending menstruation. One of the characteristic features of the secretory phase as opposed to the proliferative phase is a progressive rise in the number of apoptotic cells within endometrial glands.

The first description of apoptosis, a process associated with distinct morphologic changes and fragmentation of DNA into oligonucleosome size fragments (Kerr *et al.*, 1972) in endometrium (Figure 1) is indebted to the work of Bartelmez (1933). The identity of the type of cell death in endometrium as being apoptotic in nature was shown by Hopwood and

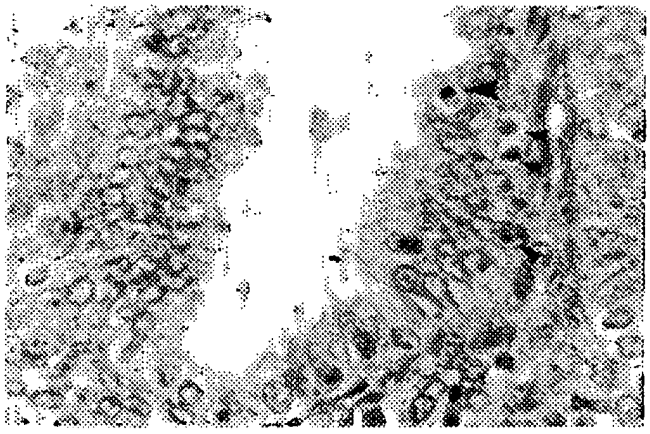

Figure 1. Apoptosis in endometrium. Apoptotic cells are readily identified in a section of human endometrium stained with haematoxylin and eosin. Apoptotic cells are found primarily underlying the epithelium and above the basement membrane (small arrowheads). An apoptotic cell is found between the epithelial cells close to the lumen (large arrowhead) (original magnification ×500).

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Levison (1975). Our investigations demonstrated that apoptosis occurs primarily during secretory/menstrual phase (Tabibzadeh *et al.*, 1994) and suggested that such apoptosis may be related to the paracrine effect of cytokines (Tabibzadeh, 1990a, 1991a, 1991b, 1994a, 1994b; Tabibzadeh *et al.*, 1994).

Apoptosis in endometrium is regulated by steroid hormones

Growth and maintenance of viability of a given cell population may depend on the presence of trophic factors. In most instances, deprivation from these trophic factors leads to an apoptotic type of cell death. For example, withdrawal from such factors as interleukin (IL)-2 (Duke and Cohen 1986; Otani *et al.*, 1993), IL-3 (Kerr *et al.*, 1972; Cohen and Duke, 1984; Otani *et al.*, 1993), IL-13 and colony-stimulating factor (CSF)-1 (Williams *et al.*, 1990) leads to an apoptotic demise of the growth factor-dependent cells. Withdrawal from the steroid hormones also results in the development of apoptosis in the steroid-sensitive tissues. The notion that deprivation from steroid hormones is implicated in apoptosis has been demonstrated in different contexts. Among the steroid hormones, perhaps oestrogen and progesterone are the principal hormones whose withdrawal leads to apoptosis in steroid sensitive tissues.

Controlled studies in animals have shown that oestrogen regulates both proliferation and apoptosis in endometrium. When administered, oestrogen is mitogenic; however, withdrawal from this steroid hormone induces apoptosis. For example, oestrogen is mitogenic to the luminal epithelial cells of the mouse uterus whereas its withdrawal leads to apoptosis in these cells (Martin and Finn, 1968, 1970; Martin *et al.*, 1973, 1976; Sandow *et al.*, 1979; Martin, 1980; Pollard *et al.*, 1987). Occurrence of apoptosis due to the withdrawal of oestrogen is not confined to the epithelium of mouse endometrium and has been observed in other species and tissues (West *et al.*, 1978; Brunner *et al.*, 1989; Kyprianou *et al.*, 1991). Cell death which was shown to be apoptotic in nature occurred in the luminal epithelium of hamster endometrium 24 h after ovariectomy (West *et al.*, 1978; Sandow *et al.*, 1979). Withdrawal from oestrogen in the immature hypophysectomized rats also resulted in apoptosis in the granulosa cells of the ovary (Billing *et al.*, 1993). Administration of diethylstilboesterol or oestradiol benzoate prevented this type of cell death (Billing *et al.*, 1993).

Ovariectomy that led to a precipitous fall in the serum progesterone concentration, resulted in apoptosis in rabbit endometrial epithelium (Rotello *et al.*, 1992). RU486 that acts as an antagonist of progesterone also led to apoptosis in these cells (Rotello *et al.*, 1992) as well as in the endometrial epithelial cells of spayed monkeys (Slayden *et al.*, 1993). These studies suggest that development of apoptosis in human endometrium may be related to oestrogen and progesterone withdrawal. However, the available evidence does not rule out the possibility that factors other than steroid hormones may also be involved in this process.

Menstruation is associated with fragmentation of endometrial glands, and loss of adhesion molecules and F-actin at the cell borders of endometrial epithelial cells

During the secretory phase of the menstrual cycle, the endometrial epithelial cells undergo secretory changes. Some view this change as a prelethal cellular state (Ferenczy, 1979). This concept is supported by the fact that the secretory epithelium loses its integrity during the menstrual phase. Detachment of endometrium is initiated in the fundus and slowly extends to the isthmus. The remainder of endometrium subsequent to this sloughing consists only of a thin tissue in the basalis and part of the surface epithelium particularly at the lower uterine segment and isthmus of the uterus (Ferenczy, 1979). What is the underlying basis for the dissolution of epithelial integrity during the menstrual phase? Epithelial cells bind together by adhesion molecules and the evidence summarized below shows that during the menstrual phase, these molecules are lost from the intercellular binding sites of the endometrial epithelium. Therefore this loss of key proteins that participate in cell–cell adhesion, may be the contributing cause for the loss of the integrity of the epithelial lining of endometrium during menstruation.

Tight, gap, intermediate (adherens) and desmosomal junctions tightly bind epithelial cells together and are responsible for the maintenance of tissue integrity (Rhodin, 1974). The proper function of each of these junctions depends on a specific set of proteins. For example, the highly related molecules desmoplakin I and II are the most abundant proteins of the cytoplasmic portion of the desmosome which consists of an electron dense, trilaminar membrane (Mueller and Franke, 1983; Kapprell *et al.*, 1988). These proteins are thought to be important in the adhesive action of the desmosomal plaque (Skerrow and Matoltsy, 1974; Mueller and Franke, 1983; Miller *et al.*, 1987; Steinberg *et al.*, 1987). The functional adherens junction is composed of a complex of proteins composed of cadherin, catenin and actin molecules (Nagafuchi and Takeichi, 1989; Ozawa *et al.*, 1989; Suzuki *et al.*, 1991; Takeichi, 1991; Geiger and Ayalon, 1992; Luna and Hitt, 1992; Mareel *et al.*, 1993). The intracytoplasmic domain of the E-cadherin molecule interacts with at least three protein species called α, β and γ catenins (Nagafuchi and Takeichi, 1989; Ozawa *et al.*, 1989; Geiger and Ayalon, 1992; Luna and Hitt, 1992; Mareel *et al.*, 1993) which in turn bind to actin filaments (Geiger and Ayalon, 1992) (Figure 2). It is not clear how the cadherin molecules associate with actin filaments; however, it has been suggested that α-catenin may fulfill the role of a cytoskeletal-linker protein. This protein shares overall structural similarity and primary amino acid sequence homology with vinculin (Herrenknecht *et al.*, 1991), a protein known to be involved in attachment of actin filaments to the plasma membrane at adhesive junctions (Otto, 1990).

Desmoplakin I/II, E-cadherin, α and β-catenins and β-actin were localized to intercellular borders as well as the luminal and basal regions of glandular epithelium (Tabibzadeh *et al.*, 1995a). This localization persisted throughout both proliferative and secretory phases (Figure 3A) (Tabibzadeh *et al.*,

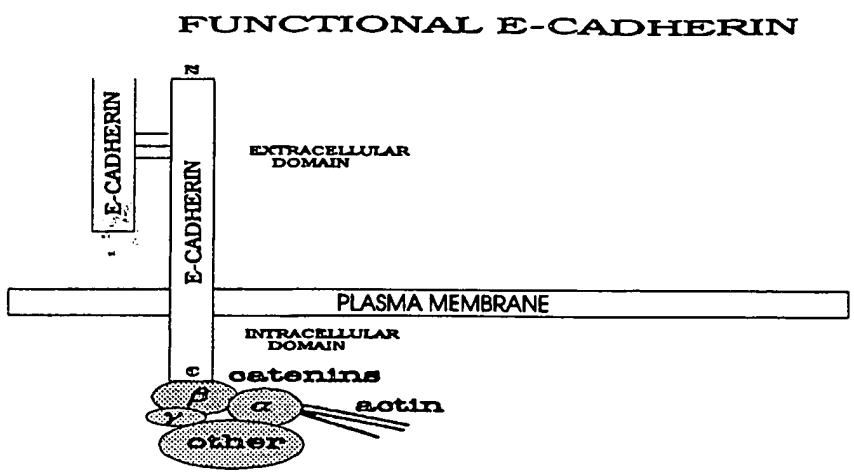

Figure 2. The molecules involved in epithelial cell–cell binding. Binding of neighbouring epithelial cells to each other is through homophilic interactions of E-cadherin molecules on two opposing plasma membranes. Function of E-cadherin molecule is regulated through its intracytoplasmic domain by interaction with catenins and with actin filaments. The opposing plasma membrane is not shown. N = N terminal, C = C terminal.

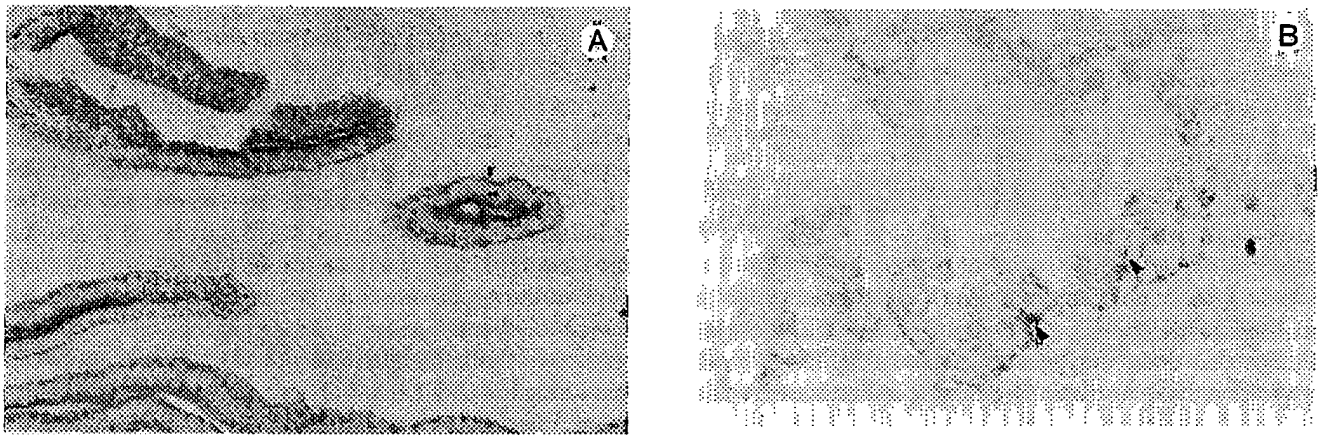

Figure 3. E-cadherin expression in proliferative and menstrual endometrium. The E-cadherin is localized immunohistochemically in the endometrial epithelial cells. (**A**) During the proliferative phase, the immunoreactive protein is orderly localized to the intercellular, luminal and basal borders (arrowheads) of cells (original magnification ×150). (**B**) During the menstrual phase, the ordered localization of E-cadherin is lost and replaced by a disorganized distribution of immunoreactive protein (arrowheads) in some cells to lack of detectable protein in other glandular cells (original magnification ×600).

1995a). Immunoreactivity of E-cadherin and α-catenin was confined to epithelial cells, whereas immunoreactive β-catenin and β-actin were present in the epithelial cells as well as the stroma and endothelial cells. F-actin was present at the intercellular borders, and the basal and luminal cytoplasm of epithelial cells in proliferative and secretory endometria. However, menstruation was associated with a number of changes in the orderly epithelial cell distribution of adhesive molecules. During this phase, desmoplakin I/II, cadherin-catenins and F-actin were lost from the cell borders (Figure 3B). Dense cytoplasmic aggregates of these molecules and their random confinement to focal intercellular binding sites became the hallmark of menstruating epithelium (Tabibzadeh *et al.*, 1995a). In addition, the cortical F-actin was lost from the epithelial cells (Tabibzadeh *et al.*, 1995a). However, these changes were not observed in the basalis region, which is not shed during the menstrual phase. Steroid hormones may play a major role in regulating the level of E-cadherin. Progesterone led to increased level of mRNA of E-cadherin in the immature mice. It was also demonstrated that, in the immature mice, 17β-oestradiol increased the mRNA level of E-cadherin in the luminal epithelium of the endometrium and surface epithelium of the ovary (Blaschuk *et al.*, 1995). Since the promoter region of the mouse E-cadherin contains progesterone and not the oestrogen response elements, the effects of oestrogen on E-cadherin mRNA expression are likely to be indirect (Blaschuk *et al.*, 1995). Whether the disturbed distribution of E-cadherin in endometrium during the menstrual phase is related to the oestrogen withdrawal remains to be demonstrated.

Menstruation is associated with compromise of vascular integrity

The structural organization of the endometrial vasculature of primates was described by Markee (1940, 1946), and Bartelmez (1957a, b). Myometrial arcuate coiled arteries give rise to a distinct microvasculature consisting of radial arteries that

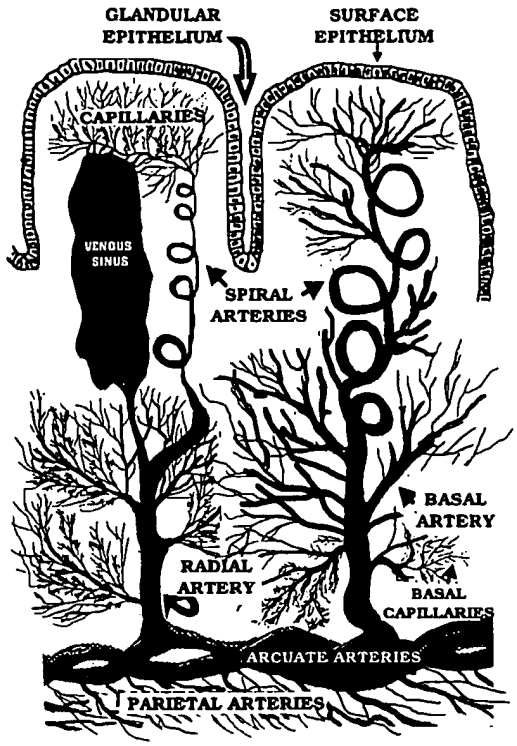

Figure 4. The structural organization of endometrial vessels. Coiled arteries in myometrium (arcuate arteries) give rise to radial arteries that branch into the basal (anastomosing) and subsequently the spiral (terminal) arteries. The spiral arteries sprout into capillaries that drain into venous sinuses.

branch into the basal (anastomosing) and subsequently the spiral (terminal) arteries. The spiral arteries sprout into capillaries that drain into venous sinuses (Figure 4). In the early proliferative phase, the sprouting spiral arteries are thin-walled and straight. Later during this phase, they become coiled and their walls increase in thickness. Postovulatory period is marked by a rapid growth of the coiled arteriolar endometrial microvasculature. However, compromise of integrity of this microvascular system first becomes noticeable in the secretory phase. Stromal oedema becomes marked on day 8 after ovulation. This minimal damage, however, passes without significant consequences until a second phase of vascular damage leads to the total loss of vascular integrity and consequent bleeding during the menstrual phase (Markee, 1940, 1946; Bartelmez, 1957a, b). The initial sign of this damage starts to appear in the late secretory phase. Fragmentations of the capillary basal lamina and marked reductions in the contacts established between the endothelial cells and pericytes are initiated in this phase of the cycle (Roberts *et al.*, 1992). During the menstrual phase, focal disruptions appear within the endothelial lined vasculature. These sites of disruptions may or may not be associated with platelets and haemostatic plugs (Christiaens *et al.*, 1980). Finally, the distal part of the arteriolar system is shed during the menstrual phase with the remaining arteriolar stumps located in the basalis being responsible for the regeneration of new coiled arteriolar system in the subsequent phase of the menstrual cycle. The injury to the endothelial cells promotes platelet aggregation, release of prostaglandin $F_2\alpha$ ($PGF_2\alpha$), thrombosis, and contraction of vessels (Srivista, 1978). The loss of functionalis, including the epithelial cells and the surrounding mantle of stroma, becomes associated with significant and continuous bleeding.

On day 3 of the menstrual cycle, for about 6–12 h, the principal mechanism for repair of the surface of the denuded endometrium is spreading and migration of epithelial cells (Ferenczy, 1976, 1979; Ferenczy *et al.*, 1979; Lindeman, 1979). Following a wave of proliferative activity in the surface epithelial cells and in stumps of the basalis glands, the integrity of endometrium is ultimately restored on day 5 of the cycle (Ferenczy, 1976, 1979; Ferenczy *et al.*, 1979; Lindeman, 1979). Concomitant with the repair of the denuded surface by proliferating epithelium, endometrial endothelium also exhibits regenerative features in the latter phase of the menstrual cycle (Ferenczy, 1976, 1979; Ferenczy *et al.*, 1979; Lindeman, 1979). Proliferation in the epithelium continues during the proliferative phase and ceases on third day post-ovulation (Tabibzadeh, 1990b).

Implication of TNF-α as a local signal contributing to the manifestations of menstruation

The notion that the characteristic phases of the menstrual cycles are induced by variation in the systemic level of oestrogen was developed by the elegant studies of Markee (1948). Whereas the withdrawal from steroid hormone seems to be the systemic signal for the induction of menstruation, the identity of the local factor(s) that within endometrium leads to the tissue shedding is still largely unrecognized. As described, menstruation carries a striking similarity to the processes of inflammation and wound repair with the exception that formation of granulation tissue or scar does not contribute to the remodelling and repair of the denuded endometrium. From this analogy, it will not be surprising to find that the cytokines known for their proinflammatory functions are involved in regulation of this process in endometrium. As indicated, menstruation is associated with distinct manifestations which include damage to the epithelium, endothelium and extracellular matrices. The effect of steroid hormone withdrawal may be mediated through the action of a local factor(s). A bona fide local 'menstruation-inducing factor' is expected to fulfil certain distinct requirements as follows: (i) the putative factor should be expressed in endometrium; (ii) production of the factor should be menstrual cycle-dependent; (iii) production of the factor should be under the regulatory control of steroid hormones; (iv) by acting on the vasculature, the factor should be able to induce oedema and haemorrhage; (v) by acting on the epithelium, the factor should be able to induce apoptosis and to alter the expression of adhesion molecules thereby facilitating the tissue shedding; (vi) by increased production or activation of the metalloproteinases, the factor should be able to induce degradation of extracellular matrices. The steroid hormone-mediated regulation of tumour necrosis factor (TNF)-α production in human endometrium has not yet been substantiated. However, the evidence presented below suggests that the expression and the known actions of TNF-α, a member of the TNF family of

molecules, fulfil all the requirements for a menstruation-inducing signal.

The TNF family of molecules consists of several proteins that exhibit considerable sequence homology (Beutler, 1992; Armitage *et al.*, 1993; Browning *et al.*, 1993; Goodwin *et al.*, 1993a,b; Smith *et al.*, 1993; Suda *et al.*, 1993; Armitage, 1994; Banchereau *et al.*, 1994). The members of this family are all type II transmembrane proteins and show homology to TNF and lymphotoxin (LT). TNF-α, the prototype of this ligand family, is a pleiotropic factor that exerts a variety of effects ranging from proinflammatory and cytotoxic, to growth and immunomodulatory on a host of different cells (Tabibzadeh, 1991b, 1994b). TNF-α is synthesized as a membrane-bound prohormone and then is proteolytically cleaved to yield a mature secreted protein with the prosequence polypeptide remaining associated with the membrane (Jue *et al.*, 1990). Human TNF-α shows 76% homology with murine TNF-α and 30% with TNF-β (Camussi *et al.*, 1991).

The receptors for TNF-α belong to a large family of molecules collectively called nerve growth factor/TNF receptor family of apoptosis inducing signals (Johnson *et al.*, 1986; Kown and Weissman, 1989; Stamenkovic *et al.*, 1989; Kohno *et al.*, 1990; Mallett *et al.*, 1990; Schall *et al.*, 1990; Smith *et al.*, 1990; Camerini *et al.*, 1991; Itoh *et al.*, 1991; Milatovich *et al.*, 1991; Durkop *et al.*, 1992; Beutler and Van Huffel, 1994). Two distinct receptors for TNF-α have been recently cloned with approximate molecular weights of 55 (TNF-R1, p55) and 75 (TNF-RII, p75) kDa (Kohno *et al.*, 1990; Schall *et al.*, 1990; Smith *et al.*, 1990). The genes for these receptors have been mapped respectively to human chromosomes 12 and 1 (Milatovich *et al.*, 1991). TNF-α induces its biological effects through interaction with both of its receptors. Receptor-specific antibodies have been used in order to demonstrate that most if not all of the biological activities of TNF-α are transduced through the TNFr-I (Englemann *et al.*, 1990; Shalaby *et al.*, 1990). However, the issue whether TNFr-I or TNFr-II is involved in the mediation of cytotoxic effect of TNF-α is hotly debated (Heller *et al.*, 1992; Tartaglia *et al.*, 1993a, b). Some believe that binding of TNF-α to either of its receptors transmits signals that lead to apoptosis (Mangan and Wahl, 1991; Higuchi and Aggarwal, 1993; Grell *et al.*, 1994). It was shown that the mitogenic or anti-proliferative effects of TNF-α on neoplastic endometrial epithelial cells depends on the dose of this cytokine (Ininns *et al.*, 1992). This effect was transmitted through the TNFr-I and not TNFr-II (Ininns *et al.*, 1992).

Endometrium encompasses leukocytes (Sen and Fox, 1967; Morris *et al.*, 1985; Kamat and Isaacson, 1987; Marshal and Jones, 1988; Tabibzadeh and Poubouridis, 1990; Tabibzadeh, 1991c) capable of production of cytokines including TNF-α. The mRNA and protein of TNF-α has been found in endometrium and both are increased during the secretory phase (Tabibzadeh, 1991c; Hunt *et al.*, 1992; Philippaeaux and Piguet, 1993; Tabibzadeh *et al.*, 1994, 1995b). The secretion of TNF-α by endometrium also exhibited a menstrual cycle dependent pattern and peaked during the menstrual phase (Tabibzadeh *et al.* 1995b). Both immunohistochemical staining and Western blot analysis failed to reveal a menstrual cycle-dependent change in the amount of TNFr-I and TNFr-II in human endometrial epithelium (Tabibzadeh *et al.*, 1995b). These studies suggest that changes in the amount of TNF-α rather than the receptors are responsible for the menstrual cycle-dependent changes induced by TNF-α in human endometrium.

A number of studies indicate that TNF-α compromises the integrity of the vessels and endometrial glands, changes that are characteristic of the menstrual phase. These data are summarized below.

TNF-α inhibits proliferation and induces apopt sis in endometrial epithelial cells

TNF-α, in a dose and time-dependent fashion, inhibited proliferation and induced apoptosis in an endometrial epithelial cell line (Tabibzadeh *et al.*, 1994). The cell death induced by TNF-α in epithelial cells was associated with the characteristic morphologic changes of apoptosis and fragmentation of DNA into oligonucleosome size fragments (Tabibzadeh *et al.*, 1994). Further analysis showed that this cytokine also induces apoptosis in glandular epithelial cells derived from normal human endometria (Tabibzadeh *et al.*, 1994). These findings suggest that cessation of proliferation of endometrial epithelium and progressive rise in apoptosis during the secretory/menstrual phases may be attributable to the increasing amount of TNF-α in endometrium during these phases of the menstrual cycle.

TNF-α induces epithelial cell–cell dissociati n (dyscohesion)

Cell–cell dissociation in endometrial epithelium is observed under two circumstances. As stated previously, dyscohesion is a requisite for menstrual shedding. On the other hand, cell–cell dissociation is seen at the point of entry of the lymphoid cells between epithelial cells. The role of TNF-α in induction of cell–cell dyscohesion has been demonstrated in various contexts. By using an endometrial epithelial cell line that formed compact spheroids *in vitro*, it was demonstrated that leukocytes induced dyscohesion of epithelium and actively entered these epithelial structures (Tabibzadeh *et al.*, 1993). The interepithelial trafficking of leukocytes was greatly facilitated by TNF-α and inhibited by an antiserum to TNF-α. Moreover, TNF-α and not other cytokines such as IL-1α, interferon (IFN)-γ or IL-6 induced epithelial dyscohesion in cohesive epithelial spheroids (Tabibzadeh *et al.*, 1993). Dyscohesion was initially associated with development of intercellular gaps and later with loss of junctions and cell–cell contacts. These findings show that leukocytes and TNF-α induce epithelial dyscohesion and that leukocytes may utilize TNF-α in order to enter epithelial layers.

TNF-α results in disordered localization of cadherin/catenin/actin complex at intercellular borders and induces conversion of F to G actin in epithelial cells

Since TNF-α impairs cell–cell binding, the adhesion molecules at the cell–cell binding sites are potential targets for its action. In epithelial cells of an endometrial cell line that actively recruited cadherin/catenin/actin to intercellular borders, TNF-α resulted in focal or virtually total loss of these proteins at cell borders (Tabibzadeh *et al.*, 1995c). However, TNF-α did not

affect the total amount of endometrial cadherin and actin (Tabibzadeh *et al.*, 1995c). Also, TNF-α, markedly and in a dose-dependent fashion, reduced the F-actin and resulted in increased amounts of globular (G) actin in endometrial epithelial cells (Tabibzadeh *et al.*, 1995c). Since the force that binds cells together is generated by the filaments of actin, conversion of F to globular (G) actin may be involved in the TNF-α mediated loss of tight epithelial cell–cell binding. This effect of TNF-α does not seem to be confined to the epithelial cells since depolymerization of F-actin by TNF-α has also been described in the endothelial cells (Goldblum *et al.*, 1993).

TNF-α compromises vascular integrity

Various lines of evidence support the concept that TNF-α compromises the vascular integrity. TNF-α, originally described as Coley's toxin, or cachectin, was identified in the late 1800s for its ability to induce haemorrhagic necrosis in tumours (Old, 1985). The identity of this factor was established in 1975, when Carswell *et al.* (1987) reported that a serum factor from animals sensitized with Bacillus Calmette-Guérin (BCG) and injected 2 weeks later with lipopolysaccharide (endotoxin) caused haemorrhagic necrosis of certain transplanted mouse tumours. The damaging effect of TNF-α on the endothelium however, does not seem to be limited to tumours and is also observed in the endothelial linings of various normal tissues including endothelium of endometrium. Tracey *et al.* (1986) reported that injection of recombinant TNF-α induces shock and tissue injury. In the lungs of TNF-α treated animals, there was evidence of oedema as well as leakage of platelets and red blood cells. Ultrastructural evaluation of the endothelium in the TNF-α treated animals showed extensive blebbing of the endothelial luminal surface and vacuolization of the cytoplasm (Remick and Kunkel, 1994). These ultrastructural changes were associated with leakage of significant amounts of fluid from vasculature (Remick and Kunkel, 1994). In another study, ultrastructural examination showed that TNF-α impaired the blood–retina barrier and induced retinal haemorrhage (Claudio *et al.*, 1994). Shalaby *et al.* (1989) showed that the endothelium of the mice uteri was particularly sensitive to the damaging effect of TNF-α. Injection of TNF-α to mice was associated with haemorrhage and apoptosis in the mouse uteri, features reminiscent of menstrual process of human endometrium. In addition, IFN-γ exhibited a synergy with TNF-α. Endothelial damage manifested in the uterus, bone marrow, liver, lungs and GI tract was enhanced when TNF-α was administered along with IFN-γ (Shalaby *et al.*, 1989). This synergism was also found with other cytokines such as IL-1 (Waage and Espevik, 1988). It was reported that the pulmonary vascular leak induced by the administration of interleukin-2 was also mediated by TNF-α (Dubinett *et al.*, 1994).

The mRNA and protein of TNF-α are regulated by steroid hormones

Various lines of evidence have shed light on the cellular and molecular events that contribute to the TNF-α induced vascular damage and consequently to oedema and haemorrhage. Under normal conditions, the endothelial cells are tightly bound together by junctions. However, TNF-α impairs this cell–cell binding, resulting in increased permeability of endothelial linings to the macromolecules and lower molecular weight solutes (Brett *et al.*, 1989). These effects of TNF-α involve the regulatory G proteins (Brett *et al.*, 1989), and are associated with developments of intercellular gaps (Brett *et al.*, 1989; Goldblum *et al.*, 1993; Kohno *et al.*, 1993). Moreover TNF-α alters cell shape and cytoskeleton, and induces conversion of F-actin to G-actin (Goldblum *et al.*, 1993). Since actin participates in cell–cell binding (Luna and Hitt, 1992), transformation of F to G-actin may be partially responsible for the loss of tight cell–cell binding as well as the increased endothelial permeability. Metalloproteinase(s) may also be involved in the TNF-α induced vascular damage. A 96 kDa gelatinolytic metalloproteinase induced by TNF-α seems to contribute to the increased microvascular endothelial permeability (Partridge *et al.*, 1993). Fibronectin, laminin, types IV and V collagens and gelatins from types I and II collagens that ensure the integrity of the basement membranes and stromal meshwork were found to be cleaved by this gelatinase. The enhanced endothelial permeability induced by TNF-α could be inhibited by tissue inhibitor of metalloproteinase (TIMP) or 1,10-phenathroline, further implicating the activity of the gelatinase induced by TNF-α in the enhanced endothelial permeability. The TNF-α mediated compromise of the vascular integrity is likely to be aggravated by apoptosis which is induced in the endothelial cells by this cytokine (Robaye *et al.*, 1991).

An important question is whether the gradual rise in the amount of TNF-α during the secretory/menstrual phases in human endometrium is regulated by steroid hormones. The available data validate the notion that steroid hormones regulate TNF-α gene expression. In peripheral blood mononuclear cells of humans, oestradiol down-regulated both the TNF-α mRNA expression (Loy *et al.*, 1992) and release of TNF-α (Ralston *et al.*, 1990). The steroid regulation of TNF-α gene expression has also been examined in the mouse endometrium (De *et al.*, 1992; Roby and Hunt, 1994). In normal mice, the TNF-α mRNA was weak to undetectable during the pro-oestrous, oestrous and dioestrous-I. However, the mRNA was detectable both in the epithelial and stromal cells during dioestrous-II (Roby and Hunt, 1994). The TNF-α protein was detectable in the epithelial cells throughout the oestrous cycle and in the stromal cells only during the dioestrous-I (Roby and Hunt, 1994). Ovariectomy, in 7 days, resulted in the loss of TNF-α mRNA and protein expression in endometrial cells (Roby and Hunt, 1994). Administration of 17β-oestradiol, progesterone or oestradiol plus progesterone resulted in the reappearance of the TNF-α mRNA and protein in epithelial and stromal cells of the mouse endometrium (De *et al.*, 1992; Roby and Hunt, 1994). The TNF-α mRNA expression in the mouse endometrium after administration of oestradiol was triphasic. The mRNA was detectable 1 and 6 h after treatment in the epithelial cells. The mRNA expression became undetectable in 24 h and reappeared 72 h later both in the epithelium and stroma (Roby and Hunt, 1994). In contrast, administration of progesterone alone or with oestradiol resulted in an extended period of TNF-α mRNA expression (Roby and Hunt, 1994). The pattern of protein immunoreactivity and bioactivity in the

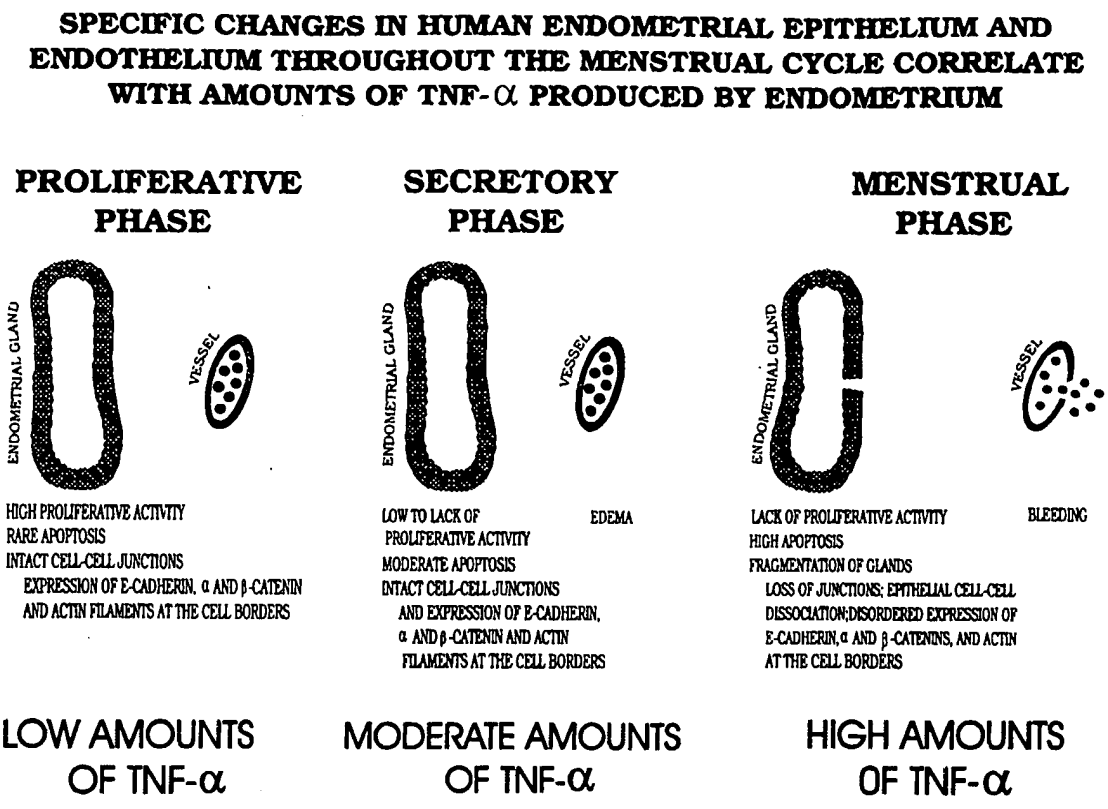

Figure 5. Correlation of attributes of endometrium with amounts of endometrial tumour necrosis factor (TNF)-α. Certain attributes characterize endometrial glands and vessels during the menstrual phase. Such attributes can be correlated with amounts of TNF-α secreted during the menstrual cycle by endometrium and its effects on endometrial epithelium and endothelium.

Table I. Correlation of effects of tumour necrosis factor (TNF)-α with the events occurring during the menstrual cycle

Event	Proliferative phase	Secretory phase	Menstrual phase	Effect of TNF-α on epithelial and endothelial cells
Amount of TNF-α (protein)	–/+	++	+++	N/A
Proliferation	+/+++[1]	+/–[1,2]	–[1]	+[1,2]
Apoptosis	–/+[1]	+/++[1]	+++[1]	+++[1,2]
Dyscohesion	–[1,2]	–[1,2]	+++[1,2]	+++[1,2]
Expression of cadherin at intercellular binding sites	+++[1]	+++[1]	+/++*[1]	+/++*[1]
Expression of catenins at intercellular binding sites	+++[1]	+++[1]	+/++*[1]	+/++*[1]
Expression of F-actin at intercellular binding sites	+++[1]	+++[1]	+/++*[1]	+/++*[1]
Oedema	–/+++	–/+++	N/A	+/+++
Haemorrhage	–	–	+++	+++[2]

Data from: Waage and Espevick, 1988; Brett *et al.*, 1989; Shalaby *et al.*, 1989; Tabibzadeh, 1990a, 1991; Kohno *et al.*, 1993; Partridge *et al.*, 1993; Robaye *et al.*, 1991; Dubinett *et al.*, 1994; Tabibzadeh *et al.*, 1994, 1995a, b, c.
Events were arbitrarily divided into: – = negative; + = low; ++ = moderate; +++ = severe; and N/A = non-applicable.
[1]Attribute of epithelium; [2]Attribute of endothelium; *Disordered expression.

endometrium of steroid-treated ovariectomized mice generally followed the pattern of mRNA expression (De *et al.*, 1992; Roby and Hunt, 1994). These findings clearly show that the TNF-α mRNA and protein expression are subject to regulation by the steroid hormones. The inference from the available data is that production of TNF-α in human endometrium may also be regulated by the steroid hormones. However, the question remains as to how this regulation takes place. In humans, the rise in the amount of TNF-α production in endometrium coincides with the late secretory/menstrual phases when systemic levels of steroid hormones are low. Therefore, it can be speculated that the TNF-α mRNA and protein expression may not only be regulated by steroid hormones but by their withdrawal as well.

As stated above, oestradiol withdrawal was associated with the development of apoptosis in hamster uterine epithelium (Sandow *et al.*, 1979), oestradiol-sensitive human mammary cancer grown in ovariectomized nude mouse (Kyprianou *et al.*, 1991), and granulosa cells in the rat ovary (Billing *et al.*, 1993). A transplantable oestrogen-dependent kidney tumour designated H301 regressed upon oestrogen withdrawal. It was demonstrated that this regression was associated with reduced mitotic activity and increased apoptosis in the tumour cells (Bursch *et al.*, 1991). Administration of RU486 which antagon-

Table II. The matrix metalloproteinase (matrixin) family

Group MMP#	Other names assigned	Sites of expression
Collagenases		
MMP-1	Interstitial collagenase EC 3.4.24.7	Stroma of endometrium, connective tissue cells
MMP-8	PMN collagenase EC 3.4.24.34	Neutrophils
Gelatinases		
MMP-2	Gelatinase A 72 kDa gelatinase Type IV collagenase EC 3.4.24.24	Most cells, stroma of endometrium
MMP-9	Gelatinase B 92 kDa gelatinase Type V collagenase EC 3.4.24.35	Stroma of endometrium, connective tissue cells, monocytes, tumour cells
MMP-7	Punctuated MP (PUMP) Matrilysin Uterine MP EC 3.4.24.23	Immature monocytes, mesangial cells, tumour cells
Stromelysins		
MMP-3	Stromelysin-1 Transin (rat) Procollagenase activator EC 3.4.24.17	Stroma of endometrium, macrophages, connective tissue cells
MMP-10	Stromelysin-2 Transin-2 (rat) EC 3.4.24.22	Stroma of endometrium
MMP-11	Stromelysin-3	Epithelium of endometrium, stromal cells in tumours
Others		
MMP-12	Metallo-elastase (mouse)	Macrophages

Data from: Woessner, 1991; Jiang and Bond, 1992.
MMP = matrix metalloproteinase; MP = metalloproteinase; PG = proteoglycan; ECM = extracellular matrix.

izes the action of progesterone also resulted in apoptosis in endometrium (Rotello *et al.*, 1992; Slayden *et al.*, 1993). Consequently, the significant increase in the apoptosis during the late secretory and menstrual phases and other specific events that characterize the menstrual phase may all be attributed to the withdrawal of steroid hormones and presumably local up-regulation of TNF-α production by endometrium. Induction of apoptosis by TNF-α in endometrial epithelial cells *in vitro* (Tabibzadeh *et al.*, 1994) and induction of apoptosis and haemorrhages in mice uteri by a combination of TNF-α and IFN-γ (Shalaby *et al.*, 1989) are consistent with this suggestion. The available evidence that is consistent with this hypothesis is summarized in Table I and portrayed in Figure 5. Clearly, other studies are required to show the regulatory roles of steroid hormones on TNF-α production. Further studies are also needed to establish the roles of TNF-α in regulation of apoptosis and in induction of manifestations of menstruation in human endometrium.

Implication of matrix metalloproteinases in menstruation

Endometrium is unique since its shedding occurs in a cyclic and coordinated fashion. The integrity of the tissues is maintained by cell–cell and cell–matrix interactions and binding as well as an intact fibrovascular meshwork. Therefore, it is logical to consider that shedding of endometrium requires participation of factors that display the ability to cause the breakdown of the cell–cell and cell–matrix adhesions and compromise the integrity of the fibrovascular stroma. The findings detailed below suggest that a group of enzymes that degrade extracellular matrices participate in the process of menstrual shedding.

Matrix metalloproteinases

Metalloproteinases (MMP) also called matrixin (Woessner, 1991), belong to the family of metalloendopeptidases. This family includes matrixin, thermolysin, astacin, serratia, and snake venom metalloproteinases (Jiang and Bond, 1992; Murphy and Docherty, 1992). At least three distinct subsets of enzymes which include the collagenases, gelatinases and stromelysins with at least nine protein members have been recognized within the matrixin family (Table II).

The enzymes within the matrixin family possess the ability to degrade the extracellular matrix components including collagens, gelatins, fibronectin, laminin, and proteoglycan. Some members also possess the additional ability to activate the latent form of other MMPs (Woessner, 1991; Ogata *et al.*, 1992). Comparison of the amino acid sequences predicted from cDNAs of MMPs reveals distinct domains that are conserved among various members of the family (Figure 6). The function of various domains is to some extent known. Signal peptide domain accounts for the direction of the translational product to the endoplasmic reticulum and ultimately its secretion. The loss of propeptide domain is associated with activation of the enzyme. The catalytic domain exhibits the ability to degrade the constituents of the extracellular matrix (ECM) after activation. The haemopexin-like domain mediates the interaction of MMPs with collagen fibres. This domain also interacts weakly with TIMP-2 when the enzyme is in inactive form and may play a role in the binding of both TIMP-1 and TIMP-2 when the enzyme becomes active (Murphy and Docherty, 1992). Deletion of the carboxy-terminal domain through either mutation or proteolysis, results in truncated enzymes. These forms of enzyme retain proteolytic activity but have alterations in substrate specificity as well as the ability to interact with the TIMP-1 and TIMP-2. Inhibition of the active forms of collagenase and gelatinase by TIMP-1 and TIMP-2 is also affected by the loss of the carboxy-terminal domain of MMPs (Kleiner and Stetler-Stevenson, 1993).

Regulatory controls over extracellular proteolysis by metalloproteinases

Control of extracellular proteolysis is critical to the maintenance of tissue integrity. Therefore several different mechanisms exist in order to exert stringent control over the degradation of the extracellular matrix by metalloproteinases. This includes control over transcription, translation, secretion and activation of metalloproteinases.

MMPs are regulated at the transcriptional level by a variety of growth factors, cytokines, oncogenes and tumour promoters (Table III). MMPs are not stored in most cell types; rather,

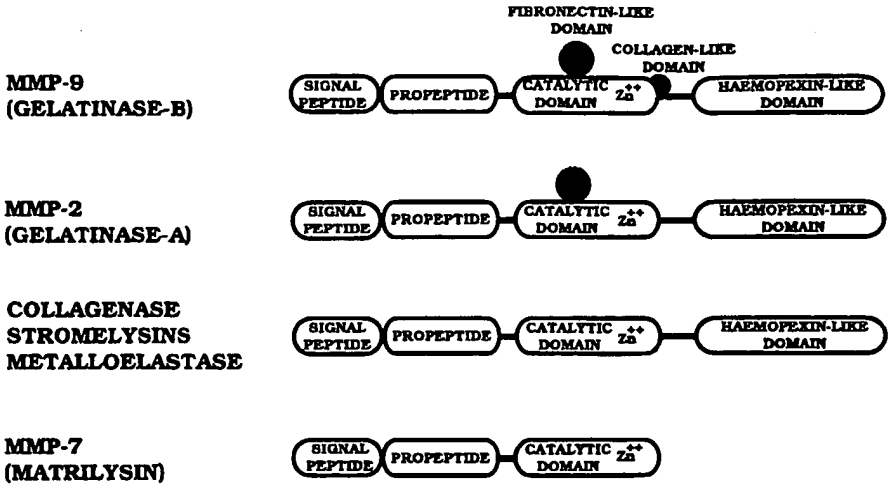

Figure 6. Various structural domains of the metalloproteinase (MMP) family. The domains within the structure of the MMP family include a signal peptide, a propeptide, a catalytic domain, a fibronectin-like domain, a collagen-like domain, and a haemopexin-like domain. However, not all MMPs contain all these domains. The gelatinases are characterized by presence of signal peptide and propeptide, catalytic, haemopexin-like and fibronectin-like domains. Gelatinase-B (MMP-9) is differentiated from gelatinase-A (MMP-2) by presence of a collagen-like domain. The collagenases and stromelysins contain the signal peptide and the propeptide, catalytic, and haemopexin-like domains and lack the fibronectin-like and collagen-like domains. The matrilysin (MMP-7) contains only the signal peptide and the propeptide, and catalytic domains.

Table III. Factors that induce/stimulate or suppress production of metalloproteinases

Inducing/stimulating factors	Suppressing factors
Cytokines	Cytokines
Tumour necrosis factor-α	Transforming growth factor-β
Interferon-α	
Interferon-β	
Interferon-γ	Steroid hormones
Interleukin-1α	Oestrogen
Interleukin-1β	Progesterone
Epidermal growth factor	Glucocorticoids
Fibroblast growth factor b	
Platelet derived growth factor	
Prostaglandin E	

Taken from Woessner, 1991.

these proteins are synthesized and secreted only after the appropriate signal has been received by the cell. Therefore, loss of the stimulating signal acts as an additional means of controlling the amount of MMPs which ultimately accounts for the degradation of the extracellular matrix of the tissues. A number of cytokines increase the production of metalloproteinases (Table III). Other factors including steroid hormones and TNF-α act to repress production of MMPs (Table III).

MMPs are secreted in a latent form (Woessner, 1991). Many different physical and chemical agents, as well as enzymes are able to induce separation of the zinc from the cysteine residue, and thereby lead to the activation of these enzymes (Vassalli *et al.*, 1991; Woessner, 1991; Murphy and Docherty, 1992; Murphy *et al.*, 1992; Gordon *et al.*, 1993). The potential physiological activators of the MMP family include plasmin, plasma kallikrein, cathepsin B, cathepsin G, and neutrophil elastase (Murphy and Docherty, 1992). Stromelysin, which is sequestered on the collagenous matrix, is particularly susceptible to activation by plasmin (Figure 7). Once activated, stromelysin induces the activation of collagenase and gelatinase-B (Figure 7) (Murphy and Docherty, 1992). On the other hand, gelatinase-A does not have any apparent cleavage site susceptible to the action of plasmin or other proteinases. The activity of this enzyme seems to depend on a self-cleavage of the pro-peptide (Nagase *et al.*, 1991; Ward *et al.*, 1991b).

The major physiological inhibitors of MMPs are shown in Table IV. They include α_2-macroglobulin, and a family of inhibitors that are collectively called tissue inhibitors of metalloproteinases (TIMP). Four different inhibitors have been reported, and three of these proteinases have been cloned and characterized (Goldberg *et al.*, 1989; Stetler-Stevenson *et al.*, 1989; Murphy and Docherty, 1992; Uria *et al.*, 1994).

Patterns of expression of MMPs in human endometrium

Presence of MMP activity in uterus was first demonstrated in the rat during postpartum period (Woessner and Taplin, 1988). The characterization of the enzyme responsible for this activity showed that it is a new member of the metalloproteinase family and was designated as uterine metalloproteinase or matrilysin (MMP-7) (Woessner and Taplin, 1988). Since tissue breakdown occurs in human endometrium during menses, many investigators have attempted to reveal presence of mRNA and secretion of a host of metalloproteinases in human endometrium particularly during the menstrual phase. Presence of MMP activity attributable to both gelatinase-A (MMP-2) and B (MMP-9) was demonstrated in human endometrium (Marbaix *et al.*, 1992). Later, presence of mRNA of both gelatinase-A (MMP-2) and B (MMP-9) as well as interstitial

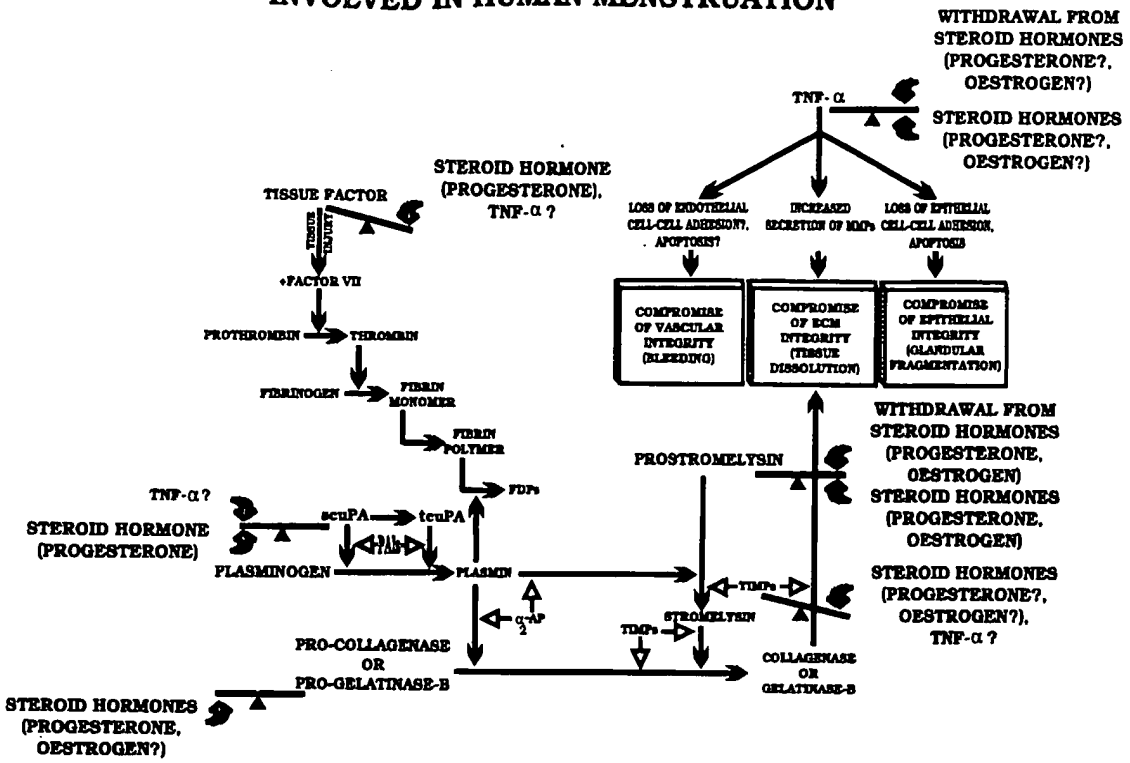

Figure 7. Potential signals and molecular pathways involved in human menstruation. The stimulatory actions are shown by solid arrows and the inhibitory actions are shown by the blank arrows. ? follows pathways demonstrated in other tissues and cells and not in endometrium thus far. Tumour necrosis factor (TNF)-α production in endometrium may be up-regulated by steroid hormone withdrawal. TNF-α leads to the compromise of integrity of vasculature, extracellular matrix (ECM) and epithelium. Tissue factor released by tissue injury forms a complex with factor VII. This complex induces conversion of prothrombin to thrombin which in turn leads to formation of fibrin from fibrinogen. Urokinase-type plasminogen activator (uPA) bound to cell surface receptors in both single chain (sc) and double-chain (dc) forms induces conversion of plasminogen to plasmin. Two inhibitors designated plasminogen activator inhibitors (PAIs) regulate this action of uPA. Plasmin results in conversion of pro-stromelysin to stromelysin, a process that is regulated by α_2-antiplasmin (α_2-AP). Plasmin and the newly generated stromelysin activate collagenase and gelatinase B. These activations are regulated by α_2-AP and by tissue inhibitors of metalloproteinases (TIMPs). The active metalloproteinases (MMPs) through degradation of the extracellular matrices compromise the integrity of vessels and ECM. Steroid hormones modulate the production of many of the signals that participate in the menstrual shedding. In some cases, this action is either stimulatory or inhibitory and in other instances the effect is mediated by steroid hormone withdrawal. TNF-α also modulates the production of various signals implicated in the menstruation.

Table IV. Inhibitors of metalloproteinases

Factor	Molecular weight (kDa)	Site
Tissue inhibitors of matrix metallo-proteinases (TIMP)		Most connective tissue cells, endometrial epihelium and stroma, synovial fluid,
TIMP-1	28/30	plasma (TIMP-1 and TIMP-2),
(TIMP-2)	21/23	breast tumour, placenta, uterus (TIMP-3)
TIMP-3	24	
α2-macroglobulin	780	Serum

Data from: Ward *et al.*, 1991a; Woessner, 1991; Murphy and Docherty, 1992; Gordon *et al.*, 1993; Denhardt *et al.*, 1993; McDonnell *et al.*, 1994; Uria *et al.*, 1994.

collagenase (MMP-1), matrilysin (MMP-7), and stromelysin-1 (MMP-3), stromelysin-2 (MMP-10) and stromelysin-3 (MMP-11) and TIMP-1 was demonstrated in human endometrium by in-situ hybridization (Rodgers *et al.*, 1994).

The patterns of expression of mRNAs of these MMPs during the menstrual cycles are different in the endometrial epithelium and stroma. Furthermore, the expression of the mRNAs of MMPs is differentially regulated throughout the menstrual cycle (Table V). The mRNA of matrilysin (MMP-7) was found primarily in the endometrial epithelium during the proliferative and menstrual phases (Matrisian *et al.*, 1994; Rodgers *et al.*, 1994;). Matrilysin protein was found in the endometrial epithelium (Rodgers *et al.*, 1993). Presence of matrilysin protein in the stroma during the menstrual phase was suggested to be due to the diffusion of protein since the mRNA of the matrilysin was absent in the stroma (Rodgers *et al.*, 1994). The expression of other MMPs was confined to the stroma. The mRNAs of stromelysin-1 (MMP-3) and stromelysin-3 (MMP-11) were found during the proliferative and menstrual phases whereas the expression of mRNA of stromelysin-2 (MMP-10) was limited to the menstrual phase (Matrisian *et al.*, 1994; Rodgers *et al.*, 1994;). The expression of mRNA of gelatinase-A (MMP-2) was found throughout the menstrual cycle whereas the expression of gelatinase-B (MMP-9) was found during the menstrual phase. The expression of TIMP-1 mRNA was found in both the stroma and epithelium, and this expression was

Table V. Menstrual cycle-dependent expression of mRNA of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human endometrium

	Menstrual phase	Proliferative phase	Secretory phase
Stroma			
MMP-1	+	+/–	+/–
MMP-2	+	+	+
MMP-3	+	+	–
MMP-9	+/–	–	–
MMP-10	+	–	–
MMP-11	+	+	–
TIMP-1	+	+	+
Epithelium			
MMP-7	+	+	–
TIMP-1	+	+/–	+/–

Data from: Rodgers *et al.*, 1994; Matrisian *et al.*, 1994.
MMP = matrix metalloproteinase; TIMP = tissue inhibitor of metalloproteinase.

amplified during the menstrual phase in the epithelium (Hampton and Salamonsen, 1994; Matrisian *et al.*, 1994; Rodgers *et al.*, 1994). The mRNA of TIMP-2 is also expressed in human endometrium throughout the menstrual cycle and its expression seems to increase during the menstrual phase (Hampton and Salamonsen, 1994). Secretion of several MMPs by the explants of endometrium and endometrial cells has been demonstrated. This includes, gelatinase-A (MMP-2) and gelatinase-B (MMP-9) (Marbaix *et al.*, 1992), stromelysin-1 (MMP-3) (Matrisian *et al.*, 1994; Schatz *et al.*, 1994), and matrilysin (MMP-7) (Matrisian *et al.*, 1994). Gelatinase-A secreted by the human stromal cells was present in the culture medium as 67 and 72 kDa species and gelatinase-B (MMP-9) was present as a homodimer with a mass of ~180 kDa (Rawdanowicz *et al.*, 1994). The expression of mRNA of the pro-MMP-1 and pro-MMP-3 has been demonstrated in the phorbol myristate acetate-stimulated stromal cells of human endometrium cultured *in vitro* (Rawdanowicz *et al.*, 1994). The release of the MMP-1 and MMP-3 by these cells into the culture medium has been confirmed by zymography and Western Blot analysis (Rawdanowicz *et al.*, 1994).

Regulation of MMPs in human endometrium

The expression of mRNA of some members of the family of MMPs varies during the menstrual cycle. The expression of interstitial collagenase (MMP-1), stromelysin-1 (MMP-3), matrilysin (MMP-7), and stromelysin-3 (MMP-11) is confined to the menstrual and proliferative phases. On the other hand, the expression of the interstitial collagenase (MMP-1), gelatinase-B (MMP-9) and TIMP-1 is primarily limited to the menstrual phase. With the exception of 72 kDa gelatinase (MMP-2), other MMPs are not expressed during the secretory phase (Table V). The cycle-dependent alterations in the amount of mRNA of MMPs suggests that the expression of MMPs is subject to regulation by steroid hormones. The hypothesis that the expression of MMPs may be positively regulated by oestrogen and negatively regulated by progesterone has been tested using endometrial explants or cells. Treatment of explant cultures of human endometrium showed that the relative amounts of matrilysin and stromelysin were not affected by oestrogen, but were significantly reduced by progesterone (Matrisian *et al.*, 1994; Osteen *et al.*, 1994). Analysis of the conditioned media of explant cultures of human endometrium by zymography revealed both the latent and active forms of interstitial collagenase (MMP-1), gelatinase-A (MMP-2) and gelatinase-B (MMP-9). In the absence of steroid hormones, the explant cultures released large amounts of enzymes. Physiological concentrations of progesterone (10–200 nM) almost completely inhibited the release of the MMP-1, total gelatinase activity and the release of the active form of MMP-9 and largely inhibited the release of the active form of MMP-2. These effects could be antagonized by the anti-progesterone, RU38486 (Marbaix *et al.*, 1992). Progesterone and not oestrogen suppressed the secretion of prostromelysin and promatrilysin from explants of human endometrium. Progesterone and not oestrogen suppressed the expression of mRNA of stromeylsin-1 and 3 and secretion of prostromelysin-1 in cultures of stromal cells of human endometrium. When epithelial cells were separated from stromal cells, the secretion of promatrilysin was not steroid-sensitive (Osteen *et al.*, 1994). However, coculture of the epithelial cells with stromal cells restored the steroidal suppression of the epithelial-specific matrilysin, suggesting the presence of a stromal cell factor(s) that may mediate the suppressive effect of progesterone (Osteen *et al.*, 1994).

Using primary cultures of stromal cells of human endometrium, it was demonstrated that oestradiol enhanced the inhibitory effect of MPA on expression of mRNA of stromelysin-1 (Schatz *et al.*, 1994). After this suppression, withdrawal of steroid hormones led to a several-fold increase in the expression of mRNA of stromelysin-1 in 4 days and stromelysin protein in 7 days after steroid hormone withdrawal (Schatz *et al.*, 1994). The secretion of stromelysin by decidual cells of the first trimester endometrium was also reduced by MPA and synergistically reduced by oestradiol plus MPA (Schatz *et al.*, 1994). Inhibition by progesterone of production and/or activation of procollagenase has been observed in many other tissues or cells. This includes postpartum rat uterine explants (Jeffrey *et al.*, 1971), smooth muscle cells (Jeffrey *et al.*, 1971), fibroblasts derived from uterine cervix of rabbit (Sato *et al.*, 1991) and guinea pig (Rajabi *et al.*, 1991) and peritoneal macrophages (Wahl, 1977).

Further analysis of progesterone-mediated regulation of human matrilysin has shown that the promoter region of the gene for this protein contains two potential glucocorticoid/progesterone regulatory elements at positions -302 and -1159 (Matrisian *et al.*, 1994). Also, the promoter region of the stromelysin has sequences that resemble the steroid-responsive elements (Frisch and Ruley, 1987). It has been demonstrated that glucocorticoids suppressed the expression of collagenase (Jonat *et al.*, 1990) and retinoic acid suppressed the expression of stromelysin (Nicholson *et al.*, 1990) through interaction with the steroid hormone receptors. In addition, progesterone had a marked inhibitory effect on the release of two plasminogen activators (tPA and uPA) from endometrial tissues in culture (Casslen *et al.*, 1986). Progesterone and oestrogen inhibited the secretion of pro-collagenase (MMP-1), pro-stromelysin-1 (MMP-3) and the steady state levels of mRNA

for these MMPs in the rabbit uterine cervical fibroblasts (Sato *et al.*, 1991). Progesterone did not increase the production of pro-gelatinase-A (MMP-2) in the same cells (Imada *et al.*, 1994). On the other hand, the secretion of TIMP-1 and TIMP-2 was increased by steroid hormone treatment (Sato *et al.*, 1991; Imada *et al.*, 1994). IL-1 enhanced the action of steroid hormones on the secretion of MMPs (Sato *et al.*, 1991). These findings show that several controlling mechanisms exist for the regulatory role that progesterone exerts on MMPs and therefore the degradation of the ECM.

It is conceivable that at least some of the actions of steroid hormones in regulating the MMP expression or secretion in endometrium may be indirect and may involve the endometrial cytokine network (Tabibzadeh, 1991b, 1994b). Regulation of the secretion of several MMPs by cytokines has been demonstrated (Table III). For example, IL-1 regulates the activity of the promoter region of stromelysin (Jonat *et al.*, 1990). The peak expressions of mRNAs of stromelysin and collagenase by fibrochondrocytes were reached respectively 8 and 24 h after stimulation by TNF-α. The enhanced secretion of both enzymes into the culture media was confirmed by Western blotting (Jasser *et al.*, 1994). The activity of these enzymes was negligible or not inducible by IGF-1, EGF, and TGF-β (Jasser *et al.*, 1994). In fibroblasts, TNF-α stimulated production of procollagenase and prostromelysin and at low doses (0.005 ng/ml) enhanced the production of TIMP. At higher doses, however, this cytokine suppressed production of TIMP in a dose-dependent manner (Ito *et al.*, 1990). Similarly, IL-1 induced the expression of mRNA of stromelysin in fibroblasts (Frisch and Ruley, 1987). It was shown that in contrast to IL-1 and TNF-α, IL-6 did not stimulate the production of collagenase nor did it stimulate the activity of these cytokines on the production of this MMP (Lotz and Guerne, 1991). However, IL-6 was found to be a strong stimulator of production of TIMP-1 in all types of connective tissue cells including fibroblasts, synoviocytes and articular chondrocytes (Lotz and Guerne, 1991). Endothelial cells did not express the mRNA for MMP-3 but expressed the mRNA for MMP-1, MMP-2 and TIMP-1 (Hanemaaijer *et al.*, 1993). TNF-α enhanced the expression of mRNA for MMP-3. TNF-α as well IL-1 enhanced the expression of MMP-9 and TIMP in these cells. MMP-2 and TIMP-2 were not affected by TNF-α or the effect of this cytokine was found to be variable (Birkedal-Hansen *et al.*, 1993). The regulation of some MMPs by the TNF-α and IL-1 in human endometrial cells has also been demonstrated. Both these cytokines, in a dose-dependent manner, stimulated the in-vitro secretion of MMP-1, MMP-3 and MMP-9 but not MMP-2 in human endometrial stromal cells (Rawdanowicz *et al.*, 1994). The lack of responsiveness of the MMP-2 to the action of the cytokines may be due to the lack of AP-1 binding site in the 5′ flanking region of the MMP-2 gene in contrast to its presence in the 5′ flanking region of the genes of MMP-1, MMP-3 and MMP-9 (Hanemaaijer *et al.*, 1993). The action of the TNF-α is not confined to its regulation of MMPs; the biosynthesis of an activator of plasminogen is also subject to regulation by TNF-α. This action of TNF-α was shown in cultured human chorionic cells (So *et al.*, 1992). TNF-α also regulates the procoagulant activity of the endothelial cells. This action of TNF-α is at least partly attributable to the increased production of tissue factor by the endothelial cells (Herbert *et al.*, 1993). The inference from these data is that cytokines including TNF-α regulate the expression and secretion of MMPs in human endometrium *in vivo*. However, the precise role that cytokines in general and TNF-α in particular play in human endometrium in controlling the action of MMPs and therefore the degradation of the extracellular matrix remains to be determined.

Other factors involved in regulation of bleeding and tissue shedding in human endometrium

The withdrawal of steroid hormones sets the stage for the secretion, and/or activation of a multiplicity of factors that participate in the bleeding associated with menstrual process. They include tissue factor (TF), plasminogen, plasminogen activators (PA), plasminogen activator inhibitors (PAI), MMPs and TIMPs. TF, a mediator of the extrinsic pathway of coagulation process, is a cell-membrane bound glycoprotein with a molecular mass of 46 kDa (Nemerson, 1988). This protein is not normally expressed by endothelial cells and is not exposed to the blood. However, once expressed and exposed to blood, it binds to factor VII, a vitamin-K dependent serine protease. The TF-VII complex then converts factor X to Xa either directly or after activation of factor IX to IXa. Finally, factor Xa converts prothrombin to thrombin which leads to the blood coagulation (Figure 7) (Nemerson, 1988). On the other hand, the substrate specific serine-proteases cleave an Arg–Val peptide bond from plasminogen and convert it to plasmin (Figure 7) (Dano *et al.*, 1985). Plasmin mediates fibrinolysis and as stated previously activates MMPs and therefore leads to degradation of the ECM. The activation of plasmin is controlled by two urokinase-type and tissue-type plasminogen activators that are designated respectively as uPA and tPA. uPA binds to a receptor that is located on the cell surface. This binding alters the activity of uPA and its susceptibility to the action of inhibitors and localizes the site of plasmin activation to the pericellular regions. The generated plasmin may itself become bound and resistant to inhibition. This cell-surface associated event, confines the action of MMPs to precisely controlled locations (Denhardt *et al.*, 1993). The activity of these activators is controlled by specific inhibitors called plasmin activator inhibitors, PAI_1 and PAI_2, which bind PAs with a high affinity (Figure 7) (Loskutoff *et al.*, 1989). Furthermore, the activity of the plasmin in activating the MMPs is inhibited by the action of α_2-antiplasmin (AP) (Figure 7).

In human endometrium, both *in vivo* and *in vitro*, the expression of TF and PAI_1 was found to accompany decidualization. Minimal endometrial staining for TF was found in the proliferative phase. The expression of TF was specifically localized in the pre-decidual cells in the secretory endometria and was most enhanced in the decidual cells in gestational endometria (Lockwood *et al.*, 1993a,b). Similarly, PAI_1 was found in the decidualized cells in the secretory phase but the staining for this protein was most pronounced in gestational endometria. In an in-vitro model of decidualization, proges-

terone increased the mRNA expression of TF and the cellular content of the protein (Lockwood *et al.*, 1993a, 1994b). Exposure of stromal cells to RU486 alone or with oestradiol plus MPA greatly reduced the levels of mRNA and protein of TF suggesting a basis for the bleeding induced by RU486 (Lockwood *et al.*, 1994a). Based on these findings it has been suggested that the fall in the amount of endometrial TF and PA-1 may make the endometrium susceptible to bleeding (Lockwood *et al.*, 1993a,b, 1994a,b). It was reported that retinoic acid counteracts both the down-regulation of thrombomodulin and the induction of tissue factor in cultured human endothelial cells exposed to tumour necrosis factor (Ishii *et al.*, 1992). In addition, retinoids regulate the mRNA expression (Turpin *et al.*, 1990) and production of TNF-α (Treisman *et al.*, 1994) and modulate the expression of its receptors (Totpal *et al.*, 1995). The receptors for retinoids are expressed in human endometrium. The mRNA of retinoic acid binding protein type I is constant throughout the menstrual cycle. On the other hand the mRNA of retinoic acid binding protein type II is high during the proliferative phase and is low during the secretory phase (Loughney *et al.*, 1995). Taken together, these lines of evidence suggest that retinoids may be amongst the important regulators of endometrial function (Loughney *et al.*, 1995). The bleeding which occurs during the menstrual phase stops within a few days. The steroid hormones through the regulation of the local factors are undoubtedly involved in this control. For example, as mentioned earlier, steroid hormones at multiple levels regulate the activity of MMPs. They also regulate the amount of MMPs as well as TIMPs. It is through regulation of these factors that the bleeding which starts upon the withdrawal of steroid hormones ultimately stops. The intricate relations between steroid hormones and the factors that participate in menstruation are illustrated in Figure 7.

Perspective

Menstrual process, a unique feature of endometrium during the reproductive cycles, is associated with exquisitely orchestrated and precisely controlled bleeding, tissue dissolution and repair. Compromise of the structural integrity of endometrium during the menstrual phase involves the vascular network, stroma and the epithelium of endometrium. Compromise of vascular integrity leads to haemorrhage. The orderly confinement of adhesive molecules to the inter-epithelial boundaries during the proliferative and secretory phases is replaced by disorganized localization of these molecules within menstruating epithelium. This is associated with loss of F-actin from the cell borders and a striking increase in apoptosis in the epithelial compartments. These events are undoubtedly essential to the glandular fragmentations that occur during menstruation. The available data suggest that withdrawal from steroid hormones is the systemic signal required for initiation of the menstruation. On the other hand, TNF-α seems to be the local factor essential or at least contributory to the diverse changes which occur within human endometrium during the menstrual phase. Although it is logical to speculate that the significant rise of endometrial TNF-α that is coincident with the menstrual phase is due to the withdrawal of steroid hormones, further studies are needed to support this hypothesis. TNF-α induces vascular damage and leads to haemorrhage. This cytokine may also be responsible for fragmentation of endometrial glands due to changes in the actin cytoskeleton and adhesion molecules of epithelial cells. Finally, TNF-α may be involved in the dissolution of stroma, glands and vasculature during the menstrual period. Some of the effect of this cytokine may be mediated by regulation of the MMPs. Activation of the plasminogen as a consequence of bleeding is responsible for the fibrinolysis as well as activation of MMPs. The activation of plasminogen is controlled by the PAIs and its activity is counteracted by α_2-AP. On the other hand, TIMPs limit the extent of degradation of the extracellular matrix by MMPs. Tissue factor also serves as part of the mechanisms that locally control the menstrual bleeding. Therefore, human endometrium is endowed with intricate and well organized systemic and local mechanisms that regulate tissue dissolution, tissue shedding and vascular bleeding during menstruation.

Acknowledgements

This work was supported by Public Health Research Grant CA 56866.

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Received on July 10, 1995; accepted on October 9, 1995